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Effect of Prolactin on Luteal Functions in the Cyclic Rat: Positive Correlation between Luteinizing Hormone-Stimulated Adenylyl Cyclase Activity and Progesterone Secretion; Role in Corpus Luteum Rescue of the Morning Surge of Prolactin on Day 3 of Pseudopregnancy*

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ABSTRACT. Rats with 4-day cycles were injected with PRL (200 μg, sc) at 12-h intervals, beginning at either 1000 or 2200 h on diestrous day 1 (D-1) or at 0600, 0800, 1000, or 1200 h on diestrous day 2 (D-2), and were killed between 0900–1000 h on proestrus (day 4 of pseudopregnancy in rats responding to treatment). To explore the effect of these treatments on luteal functions, we determined: 1) basal and LH- and isoproterenol-stimulated adenylyl cyclase activities in homogenates of dissected corpora lutea (CL) and 2) progesterone concentrations in sera.

Lack of PRL treatment resulted in rats with low serum progesterone concentrations, low LH-responsive luteal adenylyl cyclase activities, and high isoproterenol-responsive luteal adenylyl cyclase activities. Initiation of PRL treatment at 1000 or 2200 h on D-1 or at 0600 h on D-2 resulted in elevated serum progesterone, elevated response of the cyclase to LH, and somewhat reduced but still significant stimulation of the cyclase by isoproterenol. When PRL treatment was initiated at 0800 h or later on D-2, the proportion of responders decreased from 9 out of 10 in those rats with treatment beginning at 0800 h to 0 out of 5 in rats with treatment started at 1200 h. The responders had LH- and isoproterenol-stimulated cyclase activities and serum progesterone concentrations similar to those in the rats with treatment beginning at 0600 h. The nonresponders all had LH (low)- and isoproterenol (high)-stimulated adenylyl cyclase activities and serum progesterone concentrations (low) which were not significantly different from those in control animals (rats receiving no PRL).

Thus, there is a positive correlation between PRL-induced maintenance of the luteal adenylyl cyclase response to LH and the progesterone-secreting activity of CL. Luteal isoproterenol-stimulated adenylyl cyclase activity is not correlated with the functional capacity of rat CL. Finally, our data suggest that the morning PRL surge on day 3 of pregnancy or pseudopregnancy is critical for survival of CL and maintenance of progesterone secretion. (*Endocrinology* **106:** 1265, 1980)

THE CORPORA lutea (CL) of cyclic rats secrete elevated levels of progesterone on diestrous day 1 (D-1) and the first 4–5 h of diestrous day 2 (D-2). If the animals are left untreated, the CL functionally regress, as evidenced by a sharp decline in serum progesterone concentrations (1–3). CL of the cycle are rescued, however, if rats are mated or subjected to cervical stimulation between the afternoon of proestrus and the morning of estrus. This is due to the establishment of a hypothalamic clock that causes the hypophysis to secrete two surges of PRL per day for 11 or 12 days, depending on whether implantation has occurred (3–7). Smith *et al.* (3) noted that although the twice daily surges occurred continuously starting on the morning (0100–0900 h) of estrus, no significant difference in luteal function occurred until the early morning of the expected day of D-2 (day 3 of pseudopregnancy), at which time luteal progesterone production increased in pseudopregnant rats while progesterone production fell sharply in cyclic rats. Döhler and Wuttke (8) used CB-154 (2-Br-α-ergocryptine) to explore the effect on luteal function of selectively inhibiting one or more of the initial PRL surges in mated rats. Their experiments showed that blockade of the first five PRL surges (from the preovulatory proestrous surge to the afternoon surge of day 2 of expected pregnancy) did not impair the rescuing effect of the following surges of PRL. Blockade of both the morning and the evening surges on day 3 of pregnancy resulted in all of the animals aborting. Thus, their studies ascribe the rescuing function to the evening possibly also to the morning surge of PRL occurring on day 3 of pregnancy. Similarly, Smith *et al.* (9) found that injection of CB-154 at 1100 and 2300 h on D-1 (day 2 of pregnancy) caused a fall in progesterone secretion, an effect which could be prevented by simultaneous administration of PRL. Due to the use of an

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inhibitor with a stated time of effectiveness of approximately 8–10 h (8), these experiments did not permit exact delineation as to whether the morning surge was indeed required.

The purpose of the study reported below was to explore 1) whether the precise surge responsible for the rescue of the CL of the cycle could be determined by initiating the PRL surges artificially via sc injection at different times during the equivalent of days 2 and 3 of pregnancy, *i.e.* on D-1 and D-2 of a cycle, and 2) whether our previous findings (10–12) of a positive correlation between LH-stimulated adenylyl cyclase and luteal function would hold true in the face of these experimental manipulations. The results indicate that LH responsiveness of the cyclase correlates well with functional activity of CL and that the morning surge on day 3 of pseudopregnancy or pregnancy is indeed the first one required for the rescue of the CL.

Materials and Methods

Animals, treatments, and preparation of sera and homogenates

Female rats were obtained at 60 days of age from Charles River Breeding Laboratories (CD, outbred; Wilmington, MA) and were housed in air-conditioned quarters with lights on from 0600–1800 h. Food and water were available *ad libitum*. Vaginal smears were taken 7 days a week by saline lavage between 1000–1100 h. Rats were chosen for the experiment after they had shown at least two consecutive 4-day cycles. Seven groups of rats were subjected to the treatment schedules outlined in Fig. 1. At the times indicated by the *solid arrows*, PRL (200 μg; NIH-PRL-S11) was administered sc in 0.2 ml 0.9% NaCl at 12-h intervals. All rats were killed (Fig. 1, *open arrows*) by decapitation between 0900–1000 h on the day of expected proestrus (day 4 of pseudopregnancy in rats responding to treatment). Blood was collected from the trunk, allowed to clot at room temperature, and left overnight at 4–10 C. Serum was separated the next morning by centrifugation and was stored at −20 C until assayed for progesterone content within the next 6 months. Ovaries, enclosed in their respective bursae, were removed immediately after decapitation and placed into ice-cold Krebs-Ringer bicarbonate buffer prepared with half of the recommended amount of $CaCl_2$ (13). After trimming away on an ice-cold petri dish any adhering tissues, the bursa was slit open, and the CL were dissected from the intact ovary within 1–2 h. Dissections were carried out under a dissecting microscope using Graefe forceps (Roboz Surgical Co., Washington, D.C.). The CL were placed into iced Krebs-Ringer bicarbonate buffer until homogenization. Dissected CL were counted, blotted dry, weighed, homogenized as described earlier (10), and assayed immediately for adenylyl cyclase activities.

Progesterone assays

Solutions, extractions of sera and homogenates, set-up of the RIA (based on antiserum GDN 337 kindly supplied by Dr. Gordon D. Niswender), separation of free from bound progesterone, estimation of recoveries, and evaluation of results were as described in detail elsewhere (12). The characteristics of this antibody have been described by Gibori *et al.* (14) and were confirmed by us.

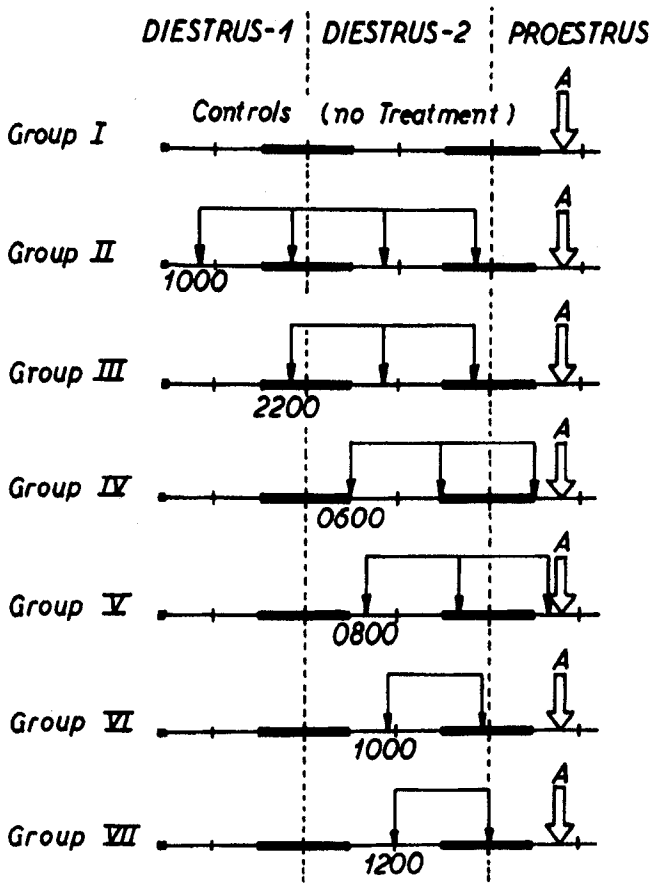

FIG. 1. Treatment schedules of rats exhibiting 4-day estrous cycles with PRL (200 μg in 0.2 ml saline, *solid arrows*). Injections (sc) were initiated at the indicated times and continued at 12-h intervals. Lighting was 12 h of light and 12 h of dark. *Open arrows* denote times at which rats were sacrificed and autopsies performed.

Adenylyl cyclase assays

Ten-microliter aliquots of luteal homogenates were assayed at 32.5 C in a final volume of 50 μl containing 3.0 mM [α-^{32}P]-ATP (5–20 × 10^6 cpm), 5.0 mM $MgCl_2$, 1.0 mM [^{3}H]cAMP (~10,000 cpm), 1.0 mM EDTA, and an ATP-regenerating system consisting of 20 mM creatine phosphate, 0.2 mg/ml creatine kinase, 0.02 mg/ml myokinase, and 25 mM Tris-HCl, pH 7.0. LH (NIH-LH-S19; 10 μg/ml) or (−)isoproterenol (a gift from Dr. F. C. Nachod, Sterling Winthrop Research Institute, Rensselaer, NY; 10^{-4} M) was added to the assay mixtures to determine cyclase activities due to hormonal stimulation. cAMP formed was isolated by a modification (15) of the method of Salomon *et al.* (16) and quantified by liquid scintillation counting.

Proteins were assayed by the method of Lowry *et al.* (17) using bovine serum albumin as standard.

Other materials

[α-^{32}P]ATP used in adenylyl cyclase assays was synthesized as described previously (18); it was supplied by the Core Laboratory on Cyclic Nucleotide Research, Center for Population Research and Studies in Reproductive Biology, Baylor College of Medicine. All other materials and sources were recently described (12).

Statistics

Statistical significance of differences between groups was calculated using analysis of variance.

Results

Effect of PRL on LH-stimulated adenylyl cyclase and correlation with effect on serum progesterone

Since in different treatment groups the basal cyclase activities varied from 5.1–8.0 pmol/mg protein·min, we have expressed LH-stimulated adenylyl cyclase activities as activities relative to basal (Fig. 2, *middle panel*). When expressed in this manner, LH stimulation of luteal adenylyl cyclase on the morning of proestrus was low in control rats. Treatment of rats with PRL starting at 1000 h on D-1 (group II) resulted in an increased ability of LH to stimulate adenylyl cyclase. While initiation of PRL treatment at 0600 h on D-2 (group IV) invariably led to elevated LH-stimulated cyclase activities, initiation of PRL treatment at later times (0800 and 1000 h; groups V and VI) led to a splitting up of groups into responders and nonresponders (9 out of 10 responded when injections were started at 0800 h on D-2, and 6 out of 8 responded when injections were started at 1000 h). None of 5 animals with PRL treatment initiated at 1200 h on D-2 (group VII) acquired LH-sensitive luteal adenylyl cyclase. Although LH-stimulated cyclase activities in responding animals with PRL treatment initiated at 0600, 0800, or 1000 h on D-2 (groups IV–VI) were significantly elevated over the cyclase activities found in control animals, they were lower than the activities found in CL of animals receiving PRL beginning at 1000 h on D-1 (group II).

Although the mean serum progesterone concentration in responding animals with PRL treatment initiated at 1000 h on D-2 (group VI) was lower than the progesterone values in animals with PRL treatment initiated at 1000 h on D-1 (group II), the mean serum progesterone concentrations in animals with PRL treatment initiated before 1000 h on D-2 (groups II–V) were not significantly different (Fig. 2, *bottom panel*).

Progesterone levels and LH-stimulated adenylyl cyclase activities in nonresponding animals were low and were not significantly different from levels found in the control group (Fig. 2; *middle and bottom panels*).

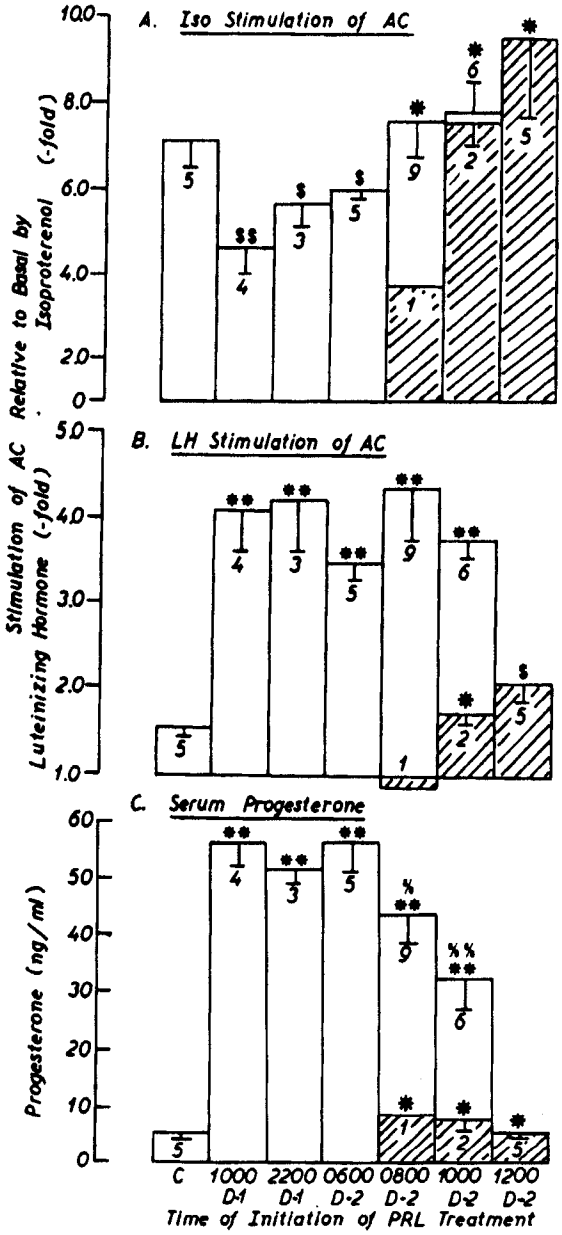

FIG. 2. Effect of PRL treatment of 4-day cyclic rats on serum progesterone and responsiveness of luteal adenylyl cyclase to LH and isoproterenol. Rats were subjected to PRL treatments initiated at differing times on D-1 or D-2, as indicated in Fig. 1. C, Control rats that received no treatment. Rats were sacrificed between 0900–1000 h on the day of the expected proestrus (day 4 of pseudopregnancy in responding rats). Sera were collected and luteal homogenates were analyzed for adenylyl cyclase activities. Relative stimulations were calculated by dividing activities obtained in the presence of hormone by the respective activities obtained in the absence of hormone. Numbers represent the numbers of animals in each group assayed for progesterone or cyclase activities. Values represent the mean ± SEM. Statistics: 1) values obtained for the experimental groups were analyzed for significance of difference when compared to values obtained for the respective control groups (*, $P > 0.1$; \$, $P > 0.05$; \$\$, $P < 0.05$; **, $P < 0.01$); 2) values obtained for experimental groups other than the one with PRL treatment initiated at 1000 h on D-1 were compared to values obtained for the respective 1000 h D-1 groups (%, $P < 0.05$; %%, $P < 0.01$).

Isoproterenol-stimulated activities

Isoproterenol-stimulated cyclase activity did not vary relative to the functional activity of CL (Fig. 2, *top panel*).

Other

Weights of CL from the different groups did not differ from one another by more than 10%.

Discussion

Progesterone secretion by CL in pregnant, pseudopregnant, and cyclic rats appears to be autonomous until the early morning hours of day 3 of pregnancy (D-2 in cyclic rats). Hypophysectomy has no apparent effects on luteal function during this time (1, 19). Moreover, the PRL surges do not alter serum progesterone concentrations, and serum LH concentrations are basal during this period (2, 3). For reasons as yet unknown, the CL switches from autonomy to PRL dependence. Based upon the data of others (3, 8, 9, 20) and the data presented here, it would appear that this transition takes place between 0600–1200 h on day 3 of pregnancy (D-2 in cyclic rats). PRL treatment initiated at 1200 h on D-2 did not rescue the CL. Therefore, it now seems certain that the morning surge (0100–0900 h) of PRL in pregnant and pseudopregnant rats is the one which is critical for luteal survival.

In previous studies in our laboratory, we have found that LH-stimulated luteal adenylyl cyclase activity is a good indicator of the functional status of CL in both rats (10–12, 21) and rabbits (22–24). The results of the current study are no exception. The most likely explanation for the increased capacity of the adenylyl cyclase of rat CL to respond to LH stimulation is the increase in LH receptor content, which has been attributed to the presence of PRL during the first several days of pregnancy and pseudopregnancy (25, 26). It is interesting to note that the earlier the initiation of PRL treatment (the longer the period of PRL treatment) in our study, the greater was the level of LH-responsive cyclase activity (approximately 31.5 pmol/min·mg protein when PRL treatment was initiated on D-1 and 22.3 pmol/min·mg protein when PRL treatment was initiated on D-2 in responders). This finding correlates well with the reported increase in LH receptor with increasing duration of exposure to PRL (25, 26).

In rat CL, the response of the adenylyl cyclase enzyme to LH develops gradually and in parallel with the capacity of CL to secrete progesterone (10–12, 21). The question arises as to whether there is a cause-effect relationship between LH-stimulated cyclase activity and progesterone secretion. Based on our findings and those of others, it would appear that a cause-effect relationship does not exist, at least not before day 8 of pregnancy or pseudopregnancy. Morishige and Rothchild (27) found that LH is not required for the maintenance of elevated serum progesterone levels until day 8 of pregnancy. Moudgal *et al.* (28) reported similar findings. In the present study, differences in serum progesterone concentrations did not always reflect a corresponding change in LH-stimulated cyclase activity. In the rabbit, the increase in luteal LH-responsive adenylyl cyclase precedes the appearance of progesterone secretion by several days (22–24). Thus, while our experiments support the idea that the presence of a LH-responsive adenylyl cyclase is indicative of luteal activity after activation of the CL (*i.e.* once the steroid-secreting machinery has been activated), the mere presence of a LH-responsive adenylyl cyclase does not correlate directly with progesterone secretion.

The physiological significance of the LH-responsive adenylyl cyclase in rats before day 8 of pregnancy or pseudopregnancy is unclear, since LH is not required before day 8 (27, 28). A correlate of this is the rabbit, which has a luteal LH-responsive cyclase but does not depend upon LH directly for luteal survival. The fact remains, however, that treatments which attenuate the LH-responsive adenylyl cyclase in CL also lead to luteolysis, *e.g.* treatment with LH or hCG (11, 22, 23), treatment with prostaglandin $F_{2\alpha}$ (29, 30), and treatment with CB-154 (Ref. 12 and the present study). These treatments may be specific for the LH-responsive adenylyl cyclase, since in the cases of LH or hCG treatment and CB-154 treatment, isoproterenol and/or prostaglandin E_1-responsive cyclase activities do not change significantly (11, 12, 22, 23). An explanation for the effect of these treatments on LH-responsive adenylyl cyclase activity could be that these treatments all affect LH receptor content or availability; LH and hCG by receptor occupancy, prostaglandin $F_{2\alpha}$ by decreasing LH receptor content (30–32), and CB-154 by blocking the PRL secretion required to increase LH receptor content (25, 26).

The reason for the presence of isoproterenol-responsive adenylyl cyclase in CL is not clear. It develops immediately after CL form, before either LH-stimulated cyclase activity or progesterone secretion begin to rise (12). Moreover, it is present after progesterone secretion and LH responsiveness have declined. Catecholamine responsiveness may be a marker for the structural integrity of CL. It is minimal in nonluteinized tissue, such as PMS gonadotropin-induced antral follicles (21), and appears to persist as long as no structural luteolysis occurs, as demonstrated in the present study (functionally inactive proestrous CL were still isoproterenol responsive). However, further studies are necessary to better characterize the functional implications of catecholamine responsiveness before it should be accepted as a luteal marker.

In summary, we have provided data that strengthen

The implication from the data of Döhler and Wuttke (8), Smith *et al.* (3, 9), and Malven (20) that the morning surge of PRL on day 3 of pregnancy is the first surge for which the CL have an absolute requirement for survival. We have also confirmed our previous findings (10–12, 21–24) that after the CL begin progesterone secretion, the presence of a luteal LH-responsive adenylyl cyclase is indicative of a functionally active CL. Yet, the physiological significance of the LH-responsive adenylyl cyclase in the CL of early pregnancy or pseudopregnancy remains unclear.

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